Appraisal of Sperm Dynamics as a Crucial Trait of Radiosterilized Spodoptera litura (Lepidoptera: Noctuidae) and Its F1 Progeny for Evaluation of the 'Inherited Sterility Technique' for Pest Suppression

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Source: Florida Entomologist, 99(sp1) : 105-118

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.sp114

Appraisal of sperm dynamics as a crucial trait of radio-sterilized *Spodoptera litura* (Lepidoptera: Noctuidae) and its F_1 progeny for evaluation of the 'inherited sterility technique' for pest suppression

Rakesh K. Seth, Zubeda Khan, Dev K. Rao and Mahtab Zarin*

Abstract

Sperm behavior represents one of the attributes in the radiogenetic technique called inherited sterility crucial for its effectiveness to suppress populations of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Quantitative and qualitative assessments of *S. litura* sperm behavior (production, descent, activation, movement and transfer to females) were made in parental (P) males that received sub-sterilizing irradiation doses of either 100 or 130 Gy and in their F_1 generation males. Age dependent production of eupyrene and apyrene spermatozoa in the testes were not affected by such irradiation in the P males, nonetheless a slight but significant effect occurred in the F_1 generation. During the rhythmic cycles of sperm descent in the photophase and scotophase, the profile and proportion of sperm descent from the testes to the upper vas deferens (UVD), seminal vesicle (SV) and duplex were not significantly influenced by irradiation in P and F_1 males. Sperm activation—assessed as percent active apyrene sperm and their intensity of motility—was not diminished in irradiated P males, while in F_1 males it showed a slight but significant reduction. Mating status was not a markedly pronounced factor in eliciting the motility of irradiated sperm. Sub-sterilized P males and their F_1 progeny were nearly as competitive as non-irradiated males in terms of sperm transfer from male to female. Successful amphimixis occurred between the altered genomes of either irradiated P males or F_1 males and the non-irradiated female genome; consequently dose dependent reductions in percent egg hatch were observed in the hatching of F_1 and F_2 eggs. Irradiation with either 100 or 130 Gy did not adversely influence the sperm characteristics in the irradiated P males and their F_1 male progeny, and this study validated the sperm' viability and performance in irradiated P males and of their F_1 sons. The findings indicated that these genetically altered sperm would fertilize the eggs of wild females and lead to effective control of this tropical pest.

Key Words: apyrene; eupyrene; common cutworm; irradiation; inherited sterility; sperm production; descent; activation; movement and transfer to females; population suppression

Resumen

El comportamiento de esperma representa uno de los atributos en la técnica radiogenética llamada esterilidad hereditaria que es crucial para su eficacia para suprimir poblaciones de *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). La evaluación cuantitativa y cualitativa del comportamiento de los espermatozoides de *S. litura* (producción, descendencia, activación, movimiento y traslado a las hembras) se hicieron en machos parientes (P_1) que recibieron una dosis de irradiación sub-esterilización de 100 ó 130 Gy y en los machos de generación F_1. La producción de espermatozoides de eupirene y apirene en los testículos dependiente a la edad no fue afectada por dicha irradiación en los machos P_1, pero un efecto leve pero significativo se produjo en la generación F_1. Durante los ciclos rítmicos de la descendencia de espermatozoides en el fotofase y escotofase, el perfil y la proporción de descendencia de espermatozoides desde los testículos a la parte superior de los vas deferens (SVD), vesícula seminal (VS) y dúplex no fueron influenciados significativamente por irradiación en P_1 y machos F_1. La activación de esperma — evaluada como el ciento de espermatozoides apirenes activos y su intensidad de movimiento no fue disminuida en los machos de P_1 irradiados, mientras que los machos de F_1 mostró una reducción leve pero significativa. El estado de apareamiento no fue un factor marcadamente pronunciado en la obtención de la movilidad de los espermatozoides irradiados. Los machos P_1 sub-esterilizados y su progenie F_1 fueron casi tan competitivos como los machos no irradiados en términos de transferencia de esperma de macho a hembra. Amfimixia exitosa ocurrió entre los genomas alterados de los machos P_1 irradiados o de machos F_1 y el genoma femenino no irradiado; en consecuencia, se observaron reducciones dependientes de dosis en cuanto del por ciento de huevos eclosionados de F_1 y F_2. La irradiación con 100 ó 130 Gy no influyó negativamente a las características de los espermatozoides en los machos de P_1 irradiados y su progenie F_1 masculina, y este estudio validó la viabilidad de los espermatozoides y el desempeño de los machos P_1 irradiados y sus hijos F_1. Los resultados indicaron que estos espermatozoides alterados genéticamente podría fertilizar los huevos de las hembras salvajes y dar lugar a un control efectivo de esta plaga tropical.

Palabras Clave: apirene; eupirene; gusano cortador común; irradiación; esterilidad heredaria; producción de esperma; descendencia; activación; movimiento y traslado a las hembras; supresión de la población

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Spodoptera litura (F.) (Lepidoptera: Noctuidae)—variously known as the oriental leafworm, common cutworm, etc.—is a notorious leaf-feeding pest of agricultural crops. It occurs in tropical and temperate regions of Asia, Australia, Europe, and Pacific islands. *Spodoptera litura* is a polyphagous and economically important pest with a wide host spectrum, including vegetables and cash crops throughout much of its established range (Chinnapandi et al. 2013). In India, *S. litura* has become a major pest and serious threat to agricultural industry due to moth's development of resistance towards commonly used insecticides (Ramakrishnan et al. 1984; Armes et al. 1997; Rame Gowda 1999; Kranthi et al. 2002). Genetic control methods can be employed as a form of biological control, which exploits the insect's mate-seeking expertise to introduce genetic abnormalities (typically, but not necessarily, dominant lethal mutations) into the wild population. The concept of controlling, managing and eliminating insect pests by manipulating their reproduction was conceived during the 1930s (Knipling 1955). Typically this involves the rearing and releasing of radiation sterilized males to introduce 1 or more dominant lethal mutations into the spermatozoa of males that are released into the field and that successfully seek out and mate with females of the pest species population. This method of pest control is commonly referred to as the sterile insect technique (SIT). However, lepidopteran insects are more radio-resistant to induction of dominant lethal mutations than most other insect orders (e.g., Diptera) and application of the high radiation dose necessary to induce complete sterility reduces competitiveness of released males. Therefore this limitation has led to a modification of the SIT for Lepidoptera, i.e., the F_1 (inherited) sterility (IS) technique in which males that have received a sub-sterilizing dose of radiation are released into fields to mate with wild females of the pest species (North 1975).

LaChance (1985) described several attributes commonly reported from studies on IS in various species of Lepidoptera. These attributes include: differential sensitivity of males and females in the parental (P) generation to radiation (usually lepidopteran females are more sensitive to radiation than males), F_1 male and female offspring exhibit substantially more sterility than the irradiated P generation, more male than female progeny are produced in the F_1 generation, the developmental time of F_1 instars may be longer, and the quality of F_1 sperm may be somewhat diminished. The mating of F_1 sterile males—which are produced in the field—with wild females enhances the efficacy of released partially sterile males, and improves the compatibility of the SIT/IS technique with other biorational pest control strategies (Carpenter et al. 2005; Seth et al. 2009). The field application of SIT/IS has been studied for many economically important lepidopteran species (North & Holt 1969; Proverbs et al. 1978; Carpenter et al. 1987; Carpenter & Gross 1993; Mastro 1993; Staten et al. 1993; Bloem et al. 1999a, b, 2001, 2004; Bloem & Bloem 2000; Carpenter et al. 2001; Hight et al. 2005).

The IS technique has been proposed for managing *S. litura* using a sub-sterilizing radiation dose of either 100 or 130 Gy for males (Seth & Sehgal 1993; Seth & Sharma 2001). When a sub-sterile male moth mates with a non-irradiated female, he transfers a full complement of sperm, but produces fewer offspring that inherit the radiation caused deleterious effects. The optimal dose of radiation for use in a program that has an IS component for lepidopteran pest control involves a trade-off between the level of sterility achieved in irradiated individuals, and the direct physiological damage leading to loss of competitiveness relative to wild individuals, both of which would increase with radiation dosage (Curtis 1985; Kean et al. 2007). Sperm dynamics is considered an important parameter with respect to the general biological quality, including the viability and competitiveness of sub-sterile parental *S. litura* males and their F_1 progeny. In this study, the influence of radiation on sperm behavior in *S. litura* was determined in terms of sperm production, sperm descent, sperm activation and sperm transfer in the sub-sterile male parents (P generation fathers) and their F_1 male progeny.

Materials and Methods

A culture of *S. litura* that originated from agricultural fields around Delhi, India was maintained in the laboratory on a chickpea (*Cicer arietinum* L.; Fabales: Fabaceae) based semi-synthetic diet as described in Seth & Sharma (2001). Laboratory environmental conditions were 27 ± 01 °C, 75 ± 5% RH, and a 12:12 h L:D photoperiod with lights on at 06.00 h and lights off at 18.00 h. Care was taken to avoid microbial infection in the culture.

Irradiation of *S. litura* was carried out in the radiobiological unit of the Institute of Nuclear Medicine and Allied Sciences (INMAS) of the Ministry of Defense in Delhi-110054. Radiation was derived from a ^{60}Co source, placed in a Gamma Cell-5000 (Board of Radiation and Isotope Technology, Trombay). The radiation dose rate of the source was between 1.26 and 1.71 KGy/h. Freshly emerged adults less than 1 d old were selected as the stage to be treated, and exposed to either 100 or 130 Gy (Seth & Sehgal 1993; Seth & Sharma 2001). Fricke dosimetry was performed on the gamma cell to establish the dose distribution to authenticate the validity of the gamma dose administered at a given dose rate.

SPERM PRODUCTION

A dissection photograph of the male reproductive tract of *S. litura* (Fig. 1) is provided to show the relationships of its components to one another. To assess sperm production and sperm release patterns from the testes, adult males were dissected at the same time each morning (10.00–11.00 h) in Belar's saline (6 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 0.2 g Na_2CO_3 and water to make 1 L) at 1 d intervals following emergence (Flint & Kressin 1969). The testis of each male was removed and placed in a 0.5 mL microcentrifuge tube and 100 µL of 2.0% lacto-aceto-orcein (LAO) was added as a specific DNA stain. The testis was macerated within the tube and the contents were thoroughly mixed by gentle vortexing. Sperm production in male lepidopterans is dimorphic, and both fertile eupyrene sperm and anucleated non-fertile apyrene sperm (Meves 1903) are transferred to the females during copulation, but only the eupyrene sperm fertilize eggs (Cook & Wedell 1996). Fertile and non-fertile sperm follow 2 distinct developmental pathways (Friedländer 1997), and are present in different ratios depending on the species. Eupyrene sperm bundles were identified by their stained nuclei, whereas apyrene sperm bundles did not take-up the stain. The average number of sperm bundles counted in 15 of such 5 µL aliquots of diluted testes extract from each sampled male was used in computing the number of sperm bundles and the 15 aliquot samples constituted 1 replicate. Twenty-five replicates (virgin males) were analyzed for each age group (0–1, 1–2, 2–3 d old) of irradiated P males, their F_1 male offspring and unirradiated P males (non-irradiated control).

SPERM DESCENT

The circadian rhythm of sperm descent from the testes down in to the reproductive tract was assessed in sub-sterile males using the method described by Seth et al. (2002b). This experiment was replicated 25 times using virgin unirradiated, irradiated and F_1 males on the 1st, 2nd and 3rd d after emergence. The numbers of eupyrene sperm bundles and individual (dissociated) apyrene sperm were quantified in 3 different parts of the reproductive tract, i.e., upper vas deferens (UVD), seminal vesicle (SV) and the ductus ejaculatorius duplex (du-

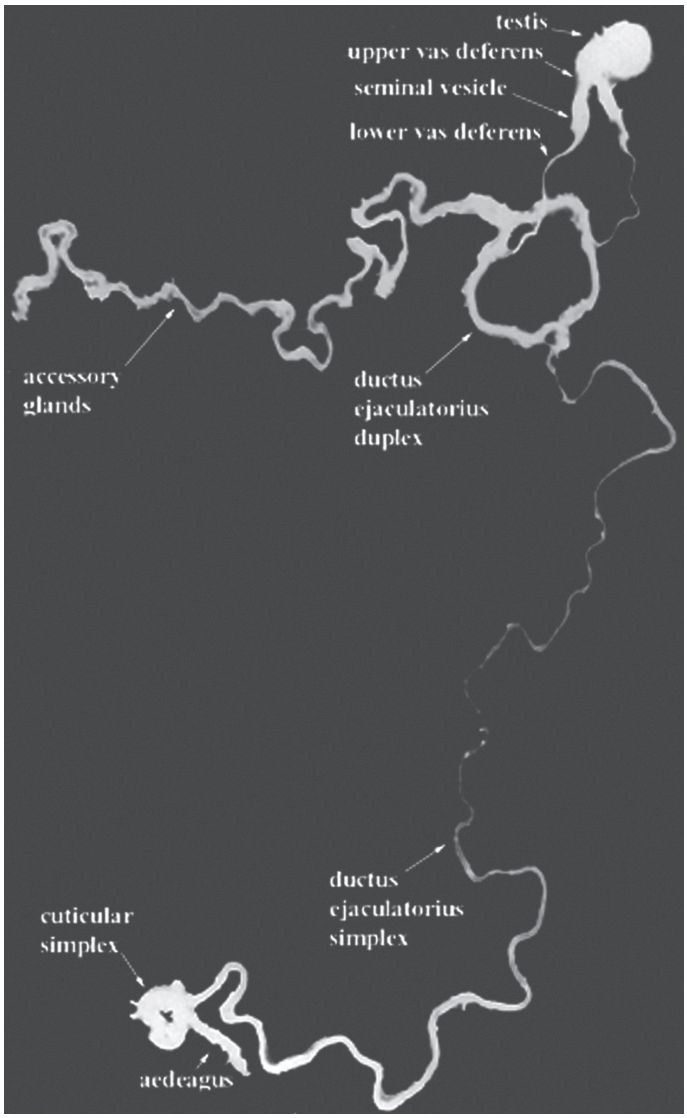

Fig. 1. Reproductive system of male moth, *Spodoptera litura*. The ductus ejaculatorius simplex is also known as the prostatic part. Sperm pass through the prostatic part at the onset of mating and acquire motility for the first time.

plex). Eupyrene sperm bundles and loose apyrene sperm were counted at 10.00–11.00 h during photophase and at 22.00–23.00 h during scotophase to ascertain the sperm descent profile and confirm their descent rhythm.

IN VITRO SPERM ACTIVATION BIOASSAY

The effect of gamma irradiation on sperm activation was studied in irradiated P males and their F_1 male progeny using 2–3 d old virgin and mated adults. An in vitro sperm bioassay was standardized (based on the procedure described by Shepherd 1974a) and used to assess the effect of gamma irradiation on sperm motility. The sperm and the activator were prepared as detailed below.

Adult males were immobilized at 4 °C in a refrigerator for 2–5 min. The males were immediately dissected in Belar's saline in a Petri plate at controlled temperature (27 ± 1 °C). The ductus ejaculatorius duplex was transected with a forceps just proximal to the prostatic part (ductus ejaculatorius simplex) and accessory glands and rinsed in 100 µL of HEPES-KOH buffer at pH 7.0 and transferred to 50 µL of 0.3 M HEPES-KOH buffer at pH 7.0 containing 20 mg/mL bovine serum albumin (BSA). Sperm with their associated secretions in HEPES-KOH buffer were then transferred to 4 cm squares of Parafilm M® placed on a wet filter paper bed in a Petri plate (5.0 cm diam) covered with a Petri plate lid and incubated at 27 ± 1 °C.

The prostatic part of 2–3 d old males was dissected out using forceps and rinsed in 0.5 mL of ammonium bicarbonate-acetic acid buffer at pH 7.0. The prostatic part of each moth was then kept in 40 µL of ammonium bicarbonate-acetic acid (0.03M) buffer at pH 7.0. The secretions oozed out into the buffer after the prostatic part was given transverse cuts. The buffer containing the prostatic secretions was centrifuged at 6,000 rpm at 4 °C for 10 min. The supernatant was collected on 4.0 cm square of Parafilm M® kept on a wet filter paper bed in a Petri-plate and incubated at 27 ± 1 °C.

Sperm Activation Assay

Sperm activation was assayed by mixing equal volumes of sperm (40 µL) and activator (40 µL) that were kept at 27 ± 1 °C, to observe the temporal profile of sperm activation. Five µL aliquots of this incubation mixture were put on a glass slide and covered with a coverslip. Observations were made under a microscope at 400 X magnification, every 5 min during the first 30 min period, every 15 min during the 30–105 min period, and every 30 min during the 105–225 min period. Parameters used for assessing spermatozoa (apyrene) activation were (i) percentage active sperm: thus each replicate constituted a mean of 10 readings (10 different visual fields on the microscopic slide having a 5 µL aliquot of incubated mixture of sperm and activator) from each male, and (ii) intensity of sperm activation: thus each replicate constituted a mean of 10 readings (of individual sperm from different visual fields) on the microscopic slide having 5 µL aliquot of incubated mixture of sperm and activator from each insect. For each data point the number of undulations per s was computed. Each observation was replicated with 25 different males. Besides these parameters, time of initiation of sperm activity, time of termination of sperm activity and duration of sperm activity—from initiation to termination of sperm activity—were also computed.

Sperm Activation in Virgin and Mated Males

Sperm Activation in Virgin Males. The effects of sub-sterilizing (100 and 130 Gy), sterilizing (200 and 250 Gy) and greater sub-lethal radiation doses (300 and 400 Gy) were examined on percent sperm activity and level of intensity of active sperm of 2–3 d old virgin P males that were irradiated as 0–1 d old males, and their F_1 progeny.

Sperm Activation in Mated Males. Irradiated 0–1 d old males were paired with non-irradiated females for 72 h and mating success was scored by assessing the presence of a spermatophore in the female's bursa copulatrix. Radiation doses were limited to the range between 100 and 250 Gy, because mating was not observed to occur when males had been treated with 300 or 400 Gy. In vitro sperm activation was also assessed in mated F_1 male adults that were descendants from 100 and 130 Gy treated male parents (P).

SPERM TRANSFER TO SPERMATHECAE

The numbers of sperm transferred from irradiated P males and from their F_1 male progeny were counted in the spermathecae of unirradiated females that had mated with either irradiated P males or non-irradiated F_1 males. Individual apyrene sperm and eupyrene sperm bundles in the spermathecae were counted between 12 and 20 h after mating with 0–1 d old males (Seth & Reynolds 1993). The 2 types of sperm were differentiated using DAPI (4, 6-diamidino-2-phenylindole-dihydrochloride) that stained distinctly the eupyrene sperm. Mating success and fertility

resulting from sperm transfer were further evaluated to correlate these parameters with the sperm transfer in spermathecae.

STATISTICAL ANALYSIS

The experiments were usually replicated 10 to 25 times and the data were subjected to one way analysis of variance (ANOVA). Percentage data was arcsine √x transformed before ANOVA, but data shown in tables and graphs were back transformed. The significance level was set at $P \leq 0.05$, and the LSD posttest was used to determine significant differences among the different treatments (Snedecor & Cochran 1989).

Results

SPERM PRODUCTION

Results indicated that the numbers of either apyrene sperm bundles or eupyrene sperm bundles in testes of 0–1 d old males were not significantly affected by the sub-sterilizing doses of either 100 or 130 Gy in comparison with non-irradiated males (Tables 1a and 1b). When 1–2 d old sub-sterilized males were evaluated, the number of apyrene bundles had been reduced by irradiation with a dose of 130 Gy ($F = 3.23$; df = 2,72; $P < 0.05$), but there was no change in the number of eupyrene sperm bundles ($F = 0.97$; df = 2,72; $P > 0.05$). In 2–3 d old males treated with 130 Gy, a 5.3% reduction in apyrene sperm bundles ($F = 3.65$, df = 2,72; $P \leq 0.05$) and an 8.4% reduction in eupyrene sperm bundles ($F = 3.54$, df = 2,72; $P \leq 0.05$) were observed in comparison with non-irradiated control males (Table 1a).

Sperm production declined in adult F_1 males derived from irradiated P male parents compared to non-irradiated P male parents. For instance, in 2–3 d old adult F_1 male offspring of males irradiated with 130 Gy, about 17% reduction in apyrene and eupyrene sperm bundles was observed in comparison with F_1 male offspring from non-irradiated P males ($F = 3.9$; df = 2,72; $P \leq 0.05$ for apyrene sperm and $F = 4.26$; df = 2,72; $P \leq 0.05$ for eupyrene sperm) (Table 1b). The ratio of eupyrene to apyrene sperm bundles was maintained at nearly the same level, i.e., 1:3, in irradiated P fathers and their F_1 sons.

SPERM DESCENT

Mating in *S. litura* was preceded by the release of sperm bundles from the testes into the paired upper vasa deferentia (UVD), followed by their transfer to the seminal vesicles (SV), with subsequent transfer to the duplex, which served as a reservoir for sperm. The effect of irradiation was assessed on the rhythmic release of sperm from the testes down to the several regions of the male reproductive tracts in P and F_1 virgin males.

Sperm Descent during the Photophase

Sperm descent during the photophase in non-irradiated males was characterized by the presence of loose apyrene sperm and eupyrene sperm bundles from the UVD to the SV where both types of the sperm were temporarily stored (Fig. 2a, b). There was no age-dependent significant difference in the numbers of the 2 sperm types in the UVD or in the SV. Thereafter, both types of sperm from the SV were transferred to the duplex where they accumulated for transfer to the female during mating (Fig 1a, b). Accumulation of sperm in the duplex increased with moth age. The pattern of sperm descent from the testes to the UVD and SV of P males treated with either 100 or 130 Gy during the photophase was similar to that of non-irradiated control males (Fig 1a, b). Increased sperm accumulation in the duplex with increasing age was likewise observed in sub-sterilized males.

Irradiation of P males decreased the number of descending loose apyrene sperm (Fig. 2a) and eupyrene sperm bundles (Fig. 2b) in comparison with the non-irradiated controls, with the magnitude of this decrease being more pronounced at 130 Gy. No significant difference was seen in the proportion of sperm descending in different regions of the reproductive tract of 0–1 d old males irradiated with either 100 or 130 Gy except a 13% reduction in eupyrene sperm bundles in the duplexes of 130 Gy treated males in comparison with the non-irradiated control males. This finding suggested that the release of sperm that might have occurred in late pupal stage was not affected by the irradiation in 0–1 d old males. Twenty four h post irradiation, a 13 and 19% reduction in the number of descending apyrene sperm was noticed in the SV of 100 and 130 Gy-treated males, respectively. The dose of 100 Gy did not affect the number of descending eupyrene sperm bundles significantly, but a dose of 130 Gy reduced the number of eupyrene sperm bundles by 38% in the UVD and by 13% in the duplexes of 1–2 d old males (Fig. 2b). In males, 2–3 d after treatment, doses of either 100 or 130 Gy resulted in reductions of 32 and 37% in apyrene sperm descent to the UVD and reductions in descent of 8 and 17% to the SV, respectively, in comparison with non-irradiated control males (Fig. 2a). The numbers of descending eupyrene sperm bundles to the UVD and the SV were not significantly affected in 2–3 d old 100 and 130 Gy-treated males.

A dose of 100 Gy had no effect on the number of apyrene sperm and eupyrene sperm bundles that accumulated in the duplex, whereas this number was reduced by 12–13% when the dose was increased to 130 Gy in comparison with non-irradiated control males (Figs. 1a, b). However, the number of descending loose apyrene sperm and bundles of eupyrene sperm was reduced in F_1 males in comparison with non-irradiated control males due to the inherited effects of irradiation of the P with either 100 or 130 Gy (Fig. 2a, b).

The temporary retention profile of apyrene sperm and eupyrene sperm bundles during the photophase in the UVD and the SV showed similar patterns in P and F_1 males in comparison with non-irradiated

Table 1a. Sperm production in the testes of unmated *Spodoptera litura* P adult males that were irradiated with either 100 or 130 Gy.

	Sperm production in different age groups of P male moths					
	Apyrene sperm bundles			Eupyrene sperm bundles		
Radiation dose given to 0–1 d old P males	0–1 d	1–2 d	2–3 d	0–1 d	1–2 d	2–3 d
0 Gy	9,162 a ± 219	8,661 a ± 227	8,343 a ± 270	3,169 a ± 162	2,848 a ± 102	2,609 a ± 58
100 Gy	8,833 a ± 202	8,246 ab ± 442	7,931 ab ± 178	2,968 a ± 103	2,737 a ± 188	2,479 ab ± 147
130 Gy	8,614 a ± 227	8,037 b ± 183	7,893 b ± 188	2,779 a ± 122	2,585 a ± 95	2,389 b ± 86
F-value	$F = 1.23$ df = 2,72	$F = 3.23$* df = 2,72	$F = 3.65$* df = 2,72	$F = 1.92$ df = 2,72	$F = 0.97$ df = 2,72	$F = 3.54$* df = 2,72

Sperm production was assessed by counting the number of eupyrene and apyrene sperm bundles in the testes of unmated adult sons. Means ± SE followed by same letter within a column are not significantly different at $P < 0.05$ level (ANOVA followed by LSD posttest); $n = 25$. *Significant at $P \leq 0.05$

Table 1b. Sperm production (apyrene and eupyrene) in testes of F_1 sons that were the offspring of P *Spodoptera litura* males irradiated with either 100 or 130 Gy.

Radiation dose given to the 0–1 d old P fathers of the adult F_1 sons	Sperm production in different age groups of adult F_1 sons of irradiated fathers					
	Apyrene sperm bundles			Eupyrene sperm bundles		
	0–1 d	1–2 d	2–3 d	0–1 d	1–2 d	2–3 d
0 Gy	9,364 a ± 164	8,920 a ± 257	8,523 a ± 213	3,103 a ± 103	2,802 a ± 67	2,654 a ± 71
100 Gy	8,191 b ± 204	7,796 b ± 239	7,450 b ± 233	2,806 ab ± 94	2,489 b ± 78	2,297 b ± 73
130 Gy	7,753 b ± 229	7,455 b ± 225	7,186 b ± 179	2,554 b ± 68	2,373 b ± 71	2,182 b ± 86
F-value	*F* = 3.81*	*F* = 4.11*	*F* = 3.90*	*F* = 3.47*	*F* = 3.42*	*F* = 4.26*
	df = 2,72	df = 2,72	df = 2,72	df = 2,72	df = 2,72	df = 2,72

Sperm production was assessed by counting the number of eupyrene and apyrene sperm bundles in the testes of unmated adult F_1 males. Means ± SE followed by same letter within a column are not significantly different at $P < 0.05$ level (ANOVA followed by LSD posttest); $n = 25$. *Significant at $P \leq 0.05$

control males (Fig. 2a, b). The reduction in the numbers of sperm descending at the duplex level was dose dependent: 4.9–12.9% in P males and 4.9–24% in F_1 males for apyrene sperm; 4.8–13.1% in P males and 8.2–23.9% in F_1 males for eupyrene sperm for the 2 doses, respectively (Fig. 2a, b).

Sperm Descent during the Scotophase

A reverse pattern of sperm descent from the testes was observed in male adults during the scotophase (22.00–23.00 h) (Fig. 2a, b). Released loose apyrene sperm and eupyrene sperm bundles were temporarily contained in the UVD during the scotophase and more sperm were found in the UVD than in the SV. No age related difference in both types of sperm was found in the UVD, whereas the number of both types of sperm changed with age in the SV ($F = 4.99$; df = 2,72; $P < 0.05$ for apyrene sperm; $F = 3.68$; df = 2.72; $P < 0.05$ for eupyrene sperm). The number of sperm that accumulated in the duplex increased with moth age (Fig. 2a, b).

During the scotophase in either 100 or 130 Gy sub-sterilized P males, there was no age related difference in the proportion of apyrene sperm and eupyrene sperm bundles present in the lumen of either the UVD, the SV or the duplex, with the exception of the number of apy-

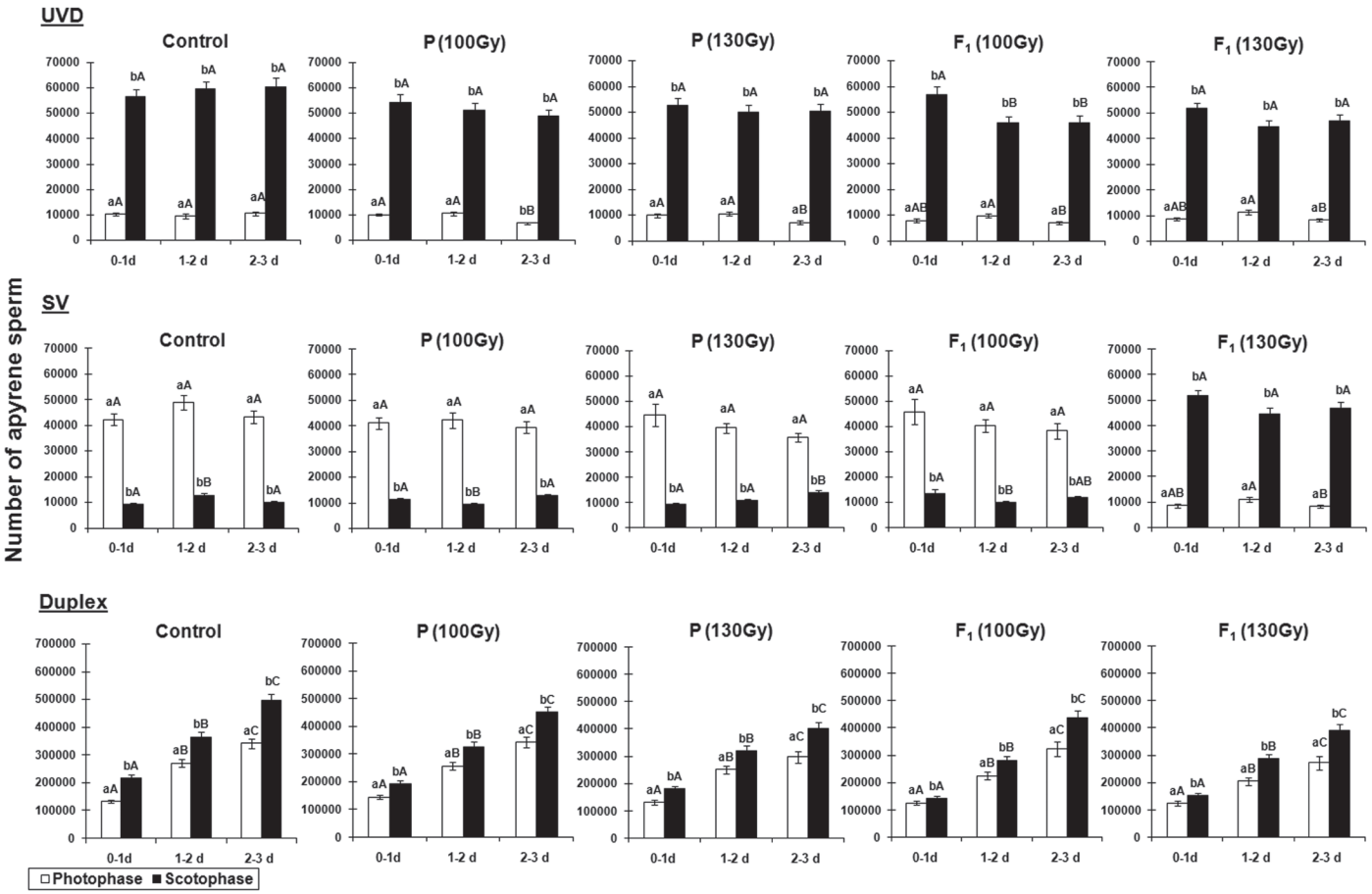

Fig. 2a. Loose apyrene sperm descent from the testes to the reproductive tract [upper vasa deferentia (UVD), seminal vesicles (SV) and the duplex] of irradiated male *Spodoptera litura* and their F_1 progeny during the photophase (white bars) and the scotophase (black bars). Means ± SE followed by the same capital letter within white bars, or within black bars for each treatment regimen of sperm descent in the UVD, SV and duplex are not significantly different at $P \leq 0.05$ (ANOVA followed by LSD post-test). Means ± SE followed by different small letter between the white bars and black bars, within each age group within a regimen are significantly different at $P \leq 0.05$ (ANOVA followed by LSD posttest).

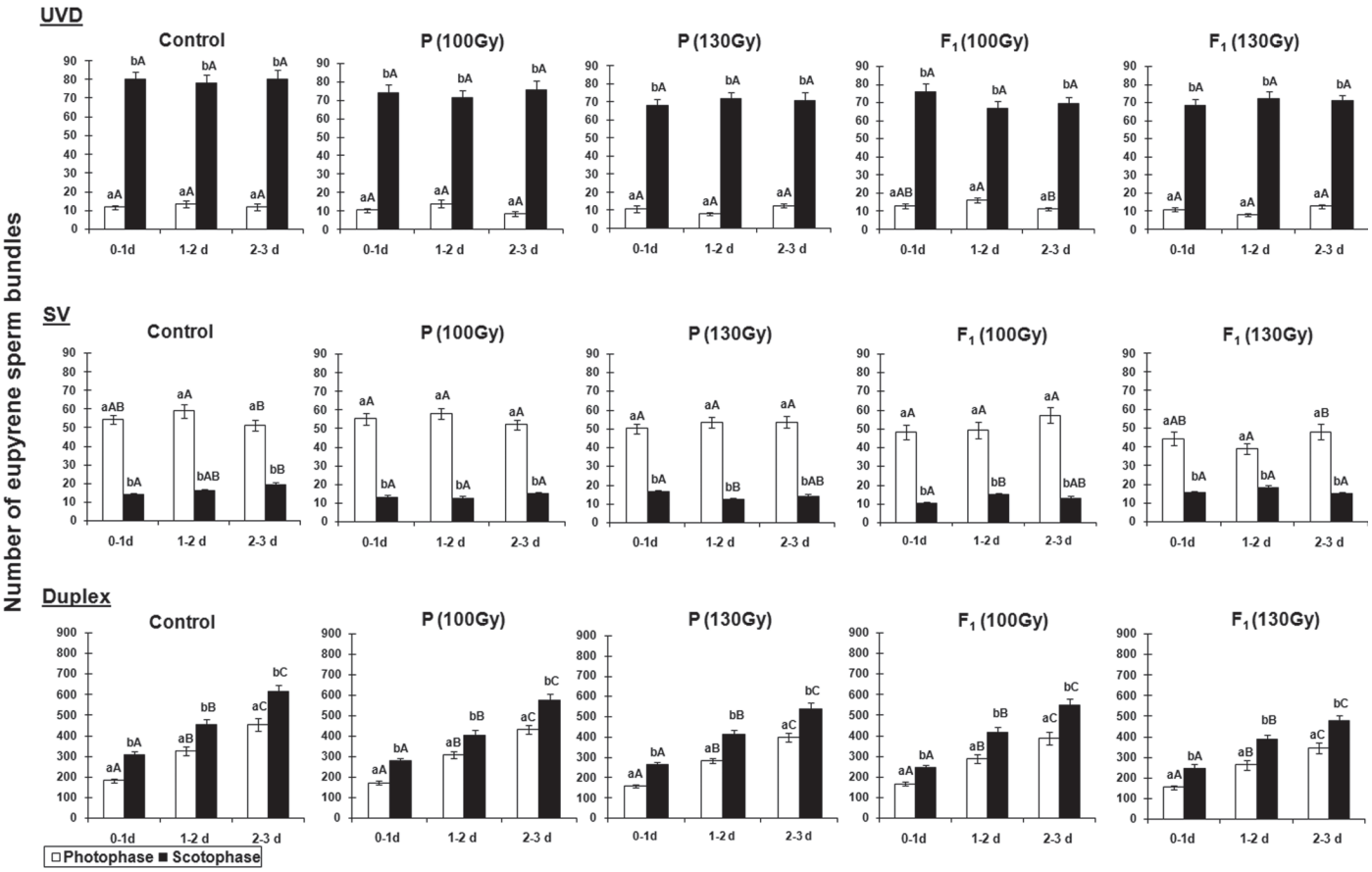

Fig. 2b. Eupyrene sperm bundles descent from the testes to the reproductive tract (upper vasa deferentia (UVD), seminal vesicles (SV) and the duplex) of irradiated male *Spodoptera litura* and their F_1 progeny during the photophase (white bars)and the scotophase (black bars). Means ± SE followed by the same capital letter within white bars, or within black bars within each treatment regimen of sperm descent in the UVD, SV and duplex are not significantly different at $P \leq 0.05$ (ANOVA followed by LSD post-test). Means ± SE followed by a different small letter between white bar and black bar, within each age group within a regimen are significantly different at $P \leq 0.05$ (ANOVA followed by LSD posttest).

rene sperm in the SV (Fig. 2a, b). As in the photophase, the number of loose apyrene sperm and bundles of eupyrene sperm that descended through the UVD and the SV to the duplex were affected in 0–1, 1–2 and 2–3 d old irradiated males during the scotophase in comparison with non-irradiated control males. A dose of 100 Gy had no effect on the number of loose apyrene sperm and bundles of eupyrene sperm that had accumulated in duplex, whereas a dose of 130 Gy resulted in a 12% decrease in these numbers in comparison with non-irradiated control males.

The numbers of loose apyrene sperm and bundles of eupyrene sperm that had descended in the duplex were not significantly different in F_1 males that were progeny of either 100 or 130 Gy treated P males in comparison with non-irradiated control males, although there were inconsistent changes in apyrene sperm number in the SV of F_1 males (Fig. 2a, b).

SPERM ACTIVATION

Sperm Activation in Virgin Adult Males

P Generation Adult Males. Apyrene sperm of non-irradiated males were not immediately active after mixing the sperm and the secretion (activator) from the prostatic part. After 5 min, 22.6% of the sperm had become active and the proportion of active sperm gradually increased with time, with peak activity of more than 75% (range of 76.1–90.8%) during 25 to 90 min. Thereafter, sperm activity gradually decreased with 57.5 and 3% of the sperm being active at 105 and 225 min, respectively (Fig. 3a).

Irradiating virgin males with 6 different doses in the range of 100–400 Gy resulted in dose dependent decreases of apyrene sperm activity at all times of observation up to 225 min in comparison with the untreated control (Fig. 3a). In vitro sperm activity studies showed that in 100–300 Gy-treated males, sperm activity became apparent within 5 min after incubation, whereas sperm of 400 Gy-treated males became active only after 10 min of incubation. The level of activation of sperm of irradiated P males varied with time, but showed a peak between 25 and 90 min of incubation (Fig. 3a).

In vitro activities of apyrene sperm of either 100 or 130 Gy-treated virgin males were similar to those of non-irradiated control males during the period between 45 and 225 min after incubation. Increasing the radiation dose to either 200 or 250 Gy resulted in complete cessation of sperm activity 225 min after the start of incubation. With a further increase of the radiation dose to the sub-lethal level, there was no more sperm activity after 135 and 180 min for doses of either 300 or 400 Gy, respectively (Fig. 3a, b).

The intensity of in vitro activity of apyrene sperm of non-irradiated males was 5.4 undulations/s after 1 min of incubation and the intensity gradually increased for another 75 min (Fig. 3b). The maximal intensity was observed during the period of 15 to 90 min after incubation, i.e., 15.3 undulations/s at 15 min and 17.6 undulations/s at 75 min, which

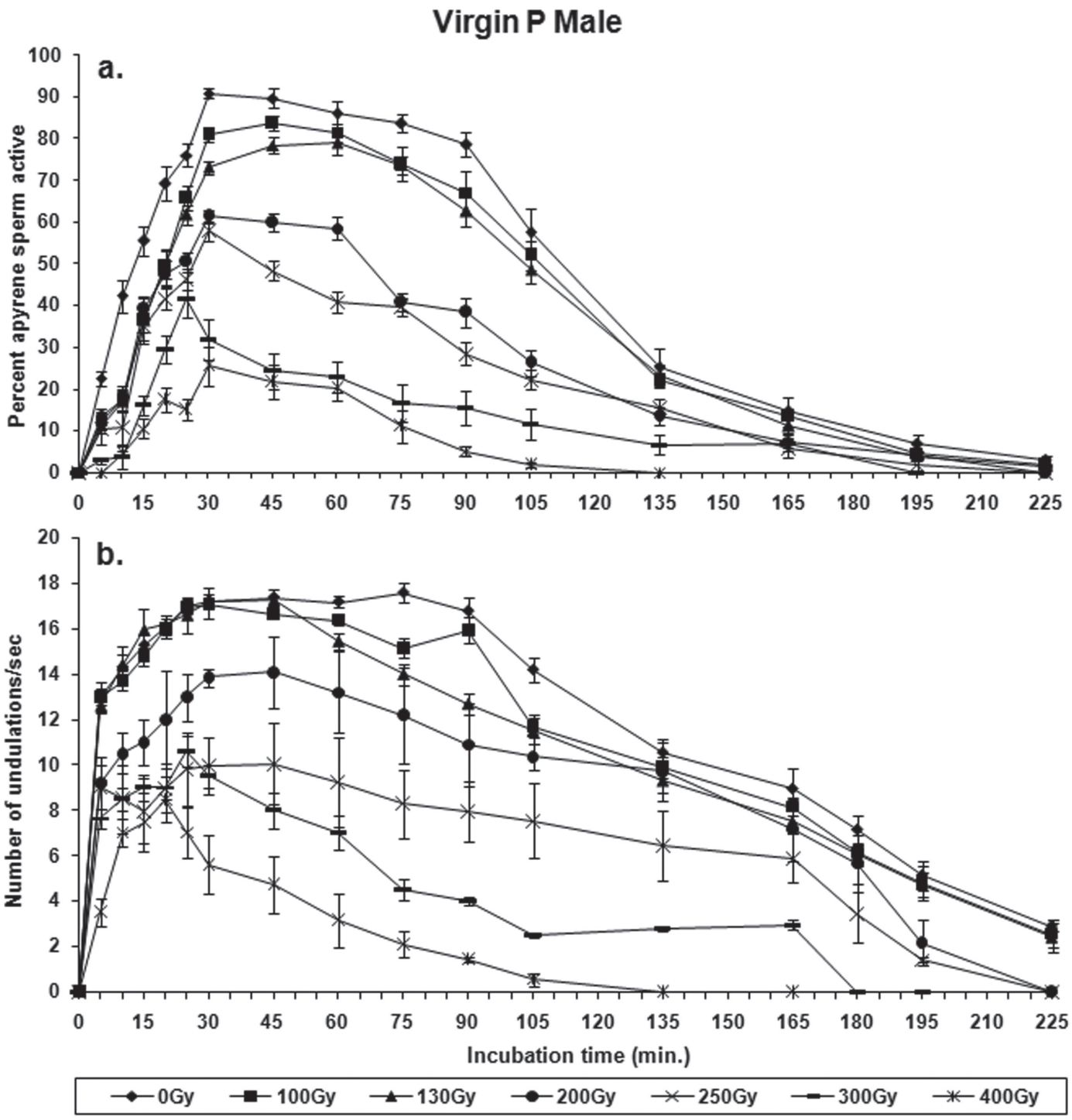

Fig. 3. Effect of gamma irradiation on (a) the percentage of active apyrene sperm, and (b) the intensity of active sperm (no. of undulations/s) in virgin irradiated parental (P) male *Spodoptera litura*.

was in accordance with the data on percentage of active sperm (> 75% sperm was active during the 25–90 min period). Thereafter, the intensity of sperm activation declined steadily with time.

The intensity of activation of apyrene sperm of virgin males irradiated with 6 different doses in the range of 100–400 Gy decreased with increasing dose, although the effect of irradiation in reducing the intensity of sperm activation was only significant with doses in the range of 200–40 0 Gy (Fig. 3b).

In either 100 or 130 Gy-irradiated P males ≥ 15 undulations/s were observed during the peak phase, which was similar to the intensity of sperm activity in non-irradiated control males. As an example, ≥ 15 undulations/s were observed during 20–90 min of incubation in the 100 Gy-treatment males but only 15–60 min of incubation in 130 Gy treatment. In males irradiated with the sterilizing doses of 200 and 250 Gy, sperm activity never reached ≥ 15 undulations/s at any of the incubation time points tested. The intensity of sperm activity gradually

decreased after the peak period until 225 min. Durations of sperm activity were found to be significantly shorter at the 2 greatest radiation doses (Fig. 3b).

F_1 Generation Adult Males. During the test period of 225 min, the percentages of apyrene sperm of F_1 adult males—progeny of P males irradiated with either 100 or 130 Gy—that showed activity in vitro were very similar to those of the P male moths irradiated with either 100 or 130 Gy. Levels of sperm activation in either P or F_1 males were not impacted by irradiation during the initial phase nor in the phase of peak activity (25–90 min). In the later phase, percentages of active sperm and durations of their activity were not significantly different from those of the non-irradiated controls (Fig 4a). In general, the effect of irradiation was more pronounced in F_1 males than in irradiated P males, although the differences often were statistically insignificant.

The intensity of apyrene sperm activation was not significantly affected at most of the time points in P males that had been irradiated with either 100 or 130 Gy nor in the corresponding F_1 adult sons in comparison with the non-irradiated controls (Fig. 4b).

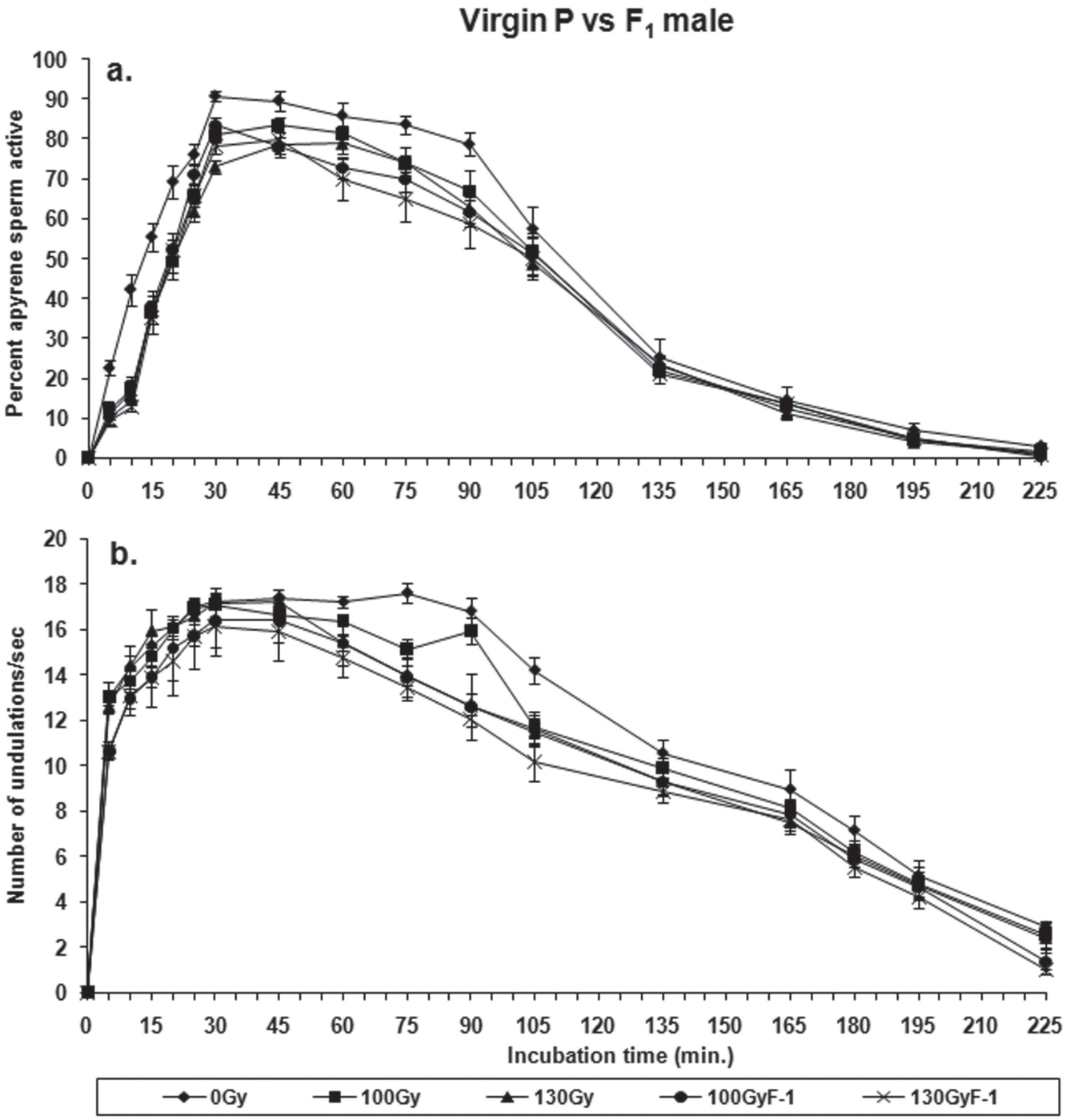

Fig. 4. Effect of gamma irradiation on (a) the percentage of active apyrene sperm and (b) the intensity of active sperm (no. of undulations /s) in virgin irradiated parental (P) male *Spodoptera litura* and their F_1progeny**.**

Sperm Activation in Mated Male Moths

P Generation Adult Males. In non-irradiated mated adult males, the period of peak activity (15–60 min after incubation) the percentage of active sperm being active was 74.6–93.3% (Fig. 5a). This was followed by a gradual decrease in active sperm with 57.0 and 1.28% of sperm being active at 105 and 225 min, respectively. In vitro activities of apyrene sperm in mated males irradiated with either 200 or 250 Gy were reduced and ceased sooner than in non-irradiated control males (Fig. 5a).

The intensity of in vitro apyrene sperm activity in non-irradiated mated P males progressively increased with a peak intensity of 16.7 undulations/s at 30 min after the start of the incubation (Fig. 5b). In mated males irradiated with either 100 or 130 Gy, the temporal profiles of intensity of apyrene sperm activation were not significantly different from those of non-irradiated controls. The intensities of sperm activity in mated males irradiated with either 200 or 250 Gy were reduced in comparison with the non-irradiated controls. The peak intensities of sperm motility in mated sub-sterilized and mated

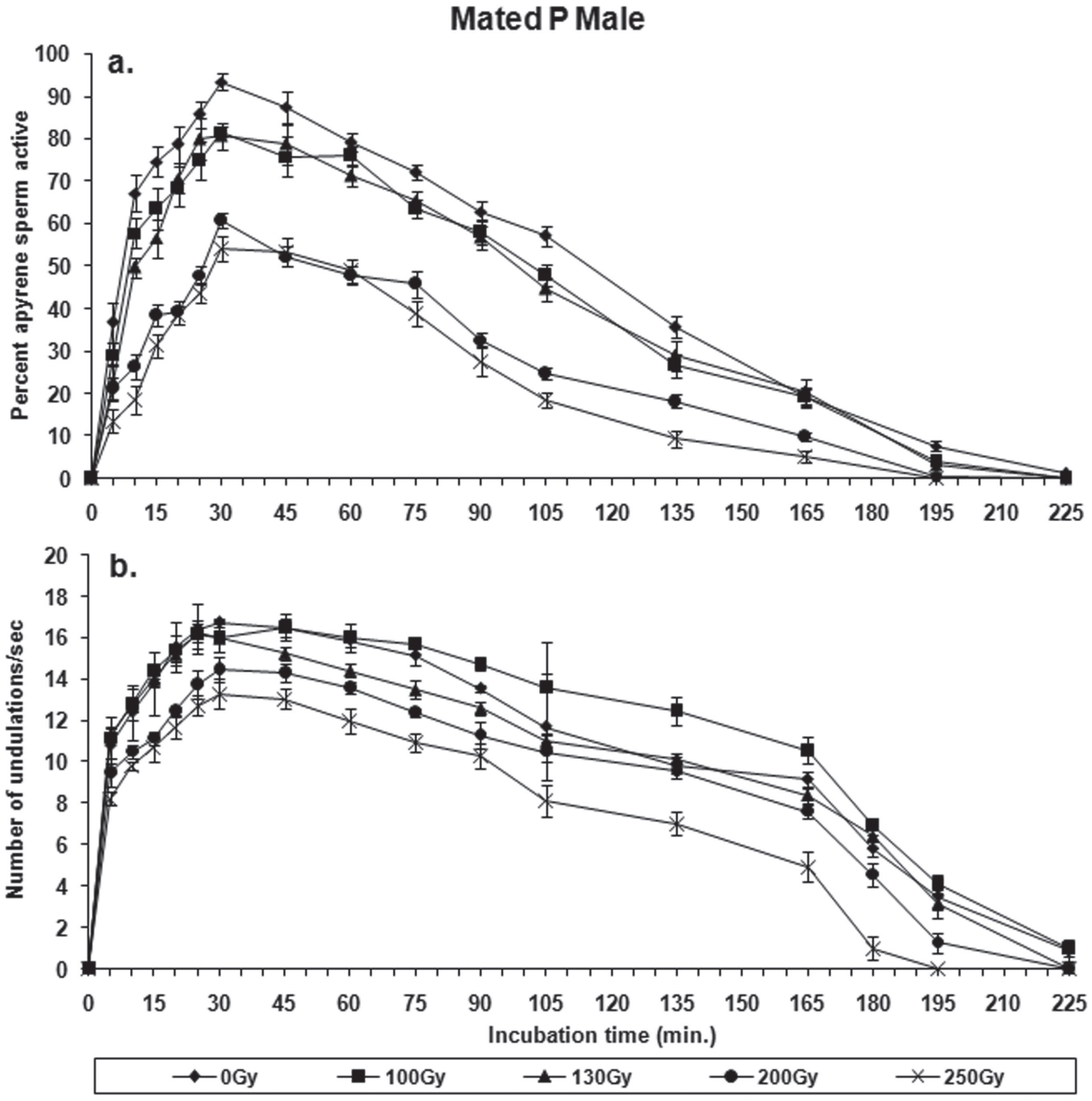

Fig. 5. Effect of gamma irradiation on (a) the percentage of active apyrene sperm, and (b) the intensity of active sperm (no. of undulations /s) in mated irradiated parental (P) male *Spodoptera litura*.

sterilized males were also reduced in comparison with irradiated virgin males (Fig. 5b).

F_1 Generation Adult Males. Levels of sperm activation in F_1 mated males that were offspring of males irradiated with either 100 or 130 Gy were reduced in comparison with mated non-irradiated control males. For instance, 15–75 min after sperm incubation, sperm activity was reduced by 18–32% and 13–56% in F_1 males that were offspring of males irradiated with either 100 or 130 Gy, respectively, in comparison with mated non-irradiated control males. The maximum proportion of motile sperm was observed at 30 min after incubation, but the proportion of active sperm was reduced in irradiated P and F_1-mated males in comparison with the mated non-irradiated control males ($F = 13.8$; df = 4,120; $P < 0.05$) (Fig. 6a).

The intensity profiles (undulations/s) of active sperm of mated F_1 males were similar to those of mated P males irradiated with 100–130 Gy and to those of mated non-irradiated control males during the first 45 min after incubation. Also the effect of irradiation was minimal in F_1 mated males in comparison with P mated males and mated non-irradiated control males during 60–90 min after incubation. In general, the intensity of sperm activity was not significantly diminished in P- and F_1-mated males in comparison to the mated non-irradiated control males (Fig. 6b).

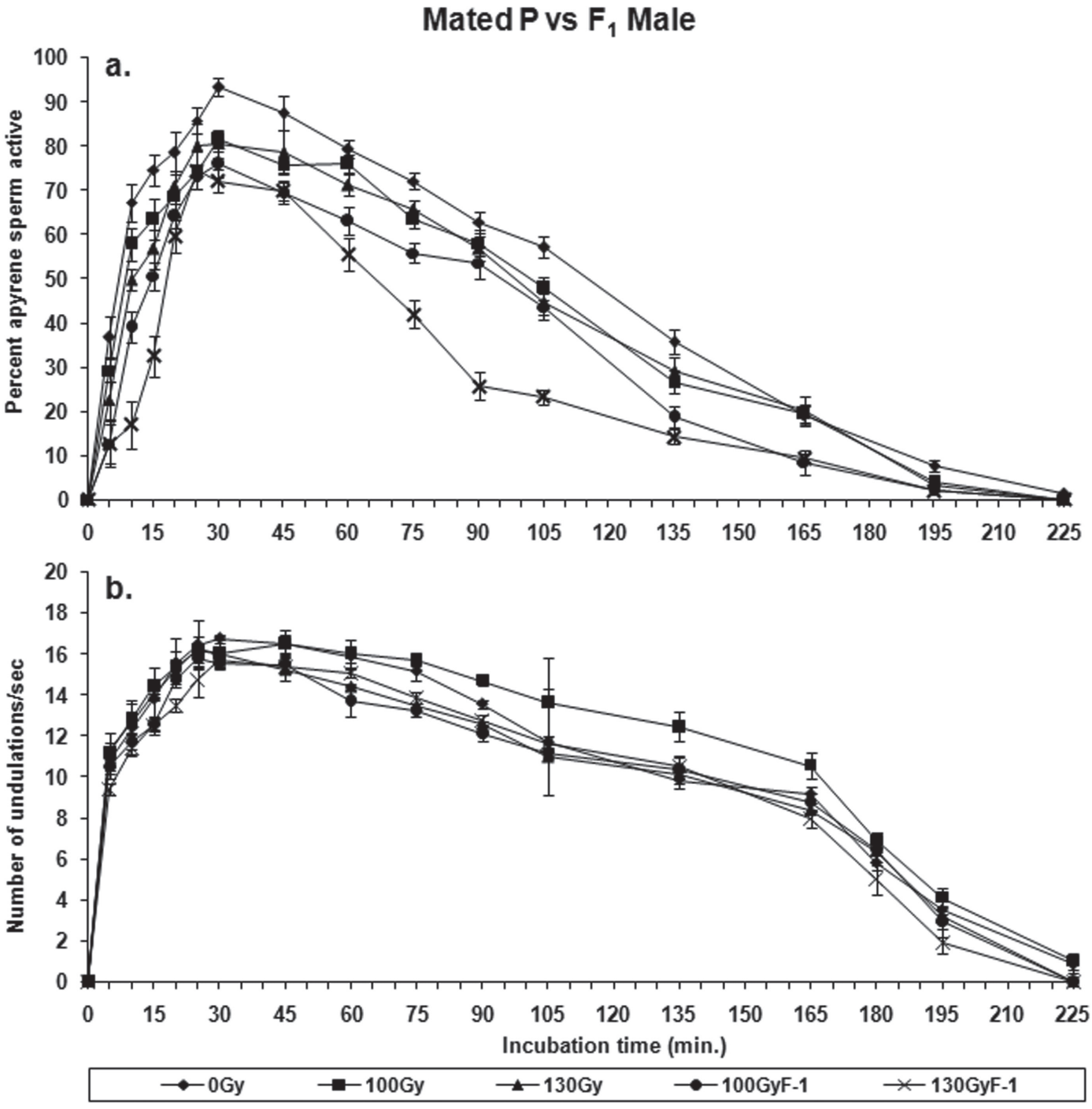

Fig. 6. Effect of gamma irradiation on (a) the percentage of active apyrene sperm, and (b) the intensity of sperm activity (no. of undulations /s) in mated irradiated parental (P) male *Spodoptera litura* and their F_1progeny**.**

SPERM TRANSFER

The total numbers of sperm transferred by irradiated P males and F_1 males to non-irradiated females were reduced in comparison with the non-irradiated control males. The numbers of sperm transferred to females mated with F_1 males—sons of fathers irradiated with either 100 or 130 Gy—were reduced by 16 and 19%, respectively, in comparison with sperm transfers by non-irradiated control males. The numbers of apyrene sperm transferred to the female spermathecae by P males irradiated with either 100 or 130 Gy and by F_1 sons derived from P fathers irradiated with 100 Gy were not significantly different from the numbers of sperm transferred by the non-irradiated control males. The number of apyrene sperm transferred by F_1 sons derived from P fathers irradiated with 130 Gy was significantly reduced by 19% in comparison with the number transferred by non-irradiated control males. No significant differences were found in the numbers of eupyrene sperm transferred by non-irradiated control males and the P males irradiated with either 100 or 130 Gy; however, the numbers of eupyrene sperm transferred to non-irradiated females by the F_1 sons of fathers irradiated with either 100 or 130 Gy were reduced by 26.7–28.1% (Table 2).

Percentages of mating success of P males irradiated with either 100 or 130 Gy that were mated with non-irradiated females decreased with increasing radiation doses; the percentages being 85.7, 78.5 and 96.4 (untreated control), respectively (Table 2). In addition percentages of mating success of their F_1 sons also mated with non-irradiated females decreased with increasing radiation doses; the corresponding percentages being 82.1, 75.6 and 96.4 (untreated control), respectively (Table 2).

Fertility levels of oviposited eggs resulting from matings of P males (irradiated with either 100 or 130 Gy) and non-irradiated females were 45.4% and 41.2% in comparison with the control level of 91.4%. In addition fertility levels of eggs resulting from matings of their F_1 sons and non-irradiated females were 26.7 and 20.9% in comparison with the control level of 91.4%.

Clearly the genetically altered sperm transferred to non-irradiated females were successfully used by females for fertilization of their eggs.

Discussion

The IS technique depends on the release in the field of sub-sterilized male insects with holocentric chromosomes, which after mating with wild females, produce sterile F_1 progeny that when mated with wild virgin females will produce unviable zygotes. The simplest models constructed to assess the effect of releasing sterile males into a natural population often assume that competitiveness ends with copulation. More sophisticated models consider frequency of successful insemination an important indicator of competitiveness, especially for lepidopteran insects (LaChance et al. 1978). In addition, a number of more subtle factors can affect the competitiveness of irradiated males and/or the efficacy of the IS technique, e.g., possible differences in radio-sensitivity of sperm located in different regions of the reproductive tract at the time of irradiation, and especially factors relating to the post-copulatory performance of the sperm in mated females, i.e., sperm dynamics, number of eggs oviposited, egg hatch, and propensity for multiple matings. The quality and quantity of sperm pertain to an area of reproductive success of male insects destined for use in the IS technique that has so far received little attention (Helinski & Knols 2009).

SPERM PRODUCTION

Our study revealed that apyrene and eupyrene sperm bundles were present in a 3:1 ratio in testes of all 3 age groups of newly emerged *S. litura* adult males. There was an age dependent reduction in the number of bundles of both types of sperm without alterations in the apyrene to eupyrene sperm bundle ratio. This temporal reduction of sperm in testes suggests daily sperm release from the testes down to the prostatic tract of *S. litura*. Helinski & Knols (2009) found that irradiation of pupae of the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae) had a negative impact on the number of sperm cells present in the testes, but such a difference was not seen for males irradiated as adults. We found that sperm production in 0–1 d old adult male *S. litura* irradiated with 100 Gy did not negatively influence sperm production during their first 3 d post-emergence, but the number of sperm was reduced in the F_1 generation. Sperm production was negatively affected to a greater extent in the 130 Gy treatment group in the F_1 generation, showing nearly a 17% reduction in the number of apyrene and eupyrene sperm. The effect of radiation administered to *S. litura* adults on sperm quantity was minimal in the P generation, but some inherited negative effects of irradiation were evident in the F_1 progeny.

SPERM DESCENT

The temporal control of sperm release appears to be important for the post-testicular maturation of sperm, and consequently for male fertility (Riemann & Giebultowicz 1991, 1992). Testicular sperm release occurs daily in a rhythm that is controlled by an endogenous circadian oscillator located in the male reproductive system (Polanska et al. 2009). This part of the work was carried out to understand the rhythmic release of sperm in *S. litura*, and to assess the effect of radia-

Table 2. Effect of gamma irradiation on the sperm transfer to the spermathecae of *Spodoptera litura* females mated with either non-irradiated or gamma-irradiated P males, or F_1 generation males; and the mating success and fertility levels of the P and F_1 males. (U is non-irradiated, IP is irradiated parent generation moth).

Gamma dose to parent male/ Nature of cross	% Mating success that resulted in sperm transfer up to spermatheca	Number of sperm transferred in spermatheca of female			Fertility (% egg hatch)
		Apyrene sperm	Eupyrene sperm	Total sperm	
0 Gy (Control) (U♀ x U♂)	96.4 a ± 2.8	42,976 a ± 1971	27,676 a ± 1451	71,751 a ± 2755	91.4 a ± 3.1
100 Gy (U♀ x IP♂)	85.7 b ± 2.9	38,580 ab ± 1471	26,088 ab ± 1464	65,345 ab ± 2434	45.4 b ± 2.7
130 Gy (U♀ x IP♂)	78.5 bc ± 3.1	38,632 ab ± 1384	24,948 abc ± 1993	64,006 abc ± 2696	41.2b ± 1.9
100 Gy (U♀ x F_1♂)	82.1 bc ± 4.1	37,876 ab ± 1281	20,274 bc ± 1551	59,798 bc ± 2960	26.7 c ± 1.4
130 Gy (U♀ x F_1♂)	75.6 c ± 3.1	33,520 b ± 1384	19,888 c ± 1190	57,445 c ± 2684	20.9 d ± 1.8
F-value	*F* = 3.10*	*F* = 22.9*	*F* = 26.3*	*F* = 35.5*	*F* = 84.4*
	df = 4,20	df = 4,120	df = 4,120	df = 4,120	df = 4,45

Means ± SE followed by same letter in a column within each regimen of a particular gamma dose among different age groups are not significantly different at $P < 0.05$ level (ANOVA followed by LSD posttest); $n = 5$ for % mating success resulting in sperm transfer up to the spermatheca, where a group of 12–15 pairs constituted each replicate; $n = 25$ for sperm transfer in mated females; $n = 10$ for fertility.

tion on the amounts of apyrene and eupyrene sperm released from the testes into the reproductive tract and the release patterns. After *S. litura* sperm were released from the testes, the eupyrene sperm remained in bundles whereas the apyrene sperm bundles dissociated into loose sperm shortly after their release from the testes (Friedländer et al. 2005).

Independently of the age of the males, an almost constant number of apyrene sperm and eupyrene sperm bundles were released and contained in the UVD at 22.00–23.00 h during the scotophase and in the SV at 10.00–11.00 h during the photophase. This pattern of sperm release might be related to some histological mechanisms (Giebultowicz et al. 1996, Bebas et al. 2002a, b), which would regulate the release of a specific number of sperm that are then retained in different parts of the reproductive tract. There was an age dependent increase in the number of both sperm types in a constant proportion in the duplex, suggesting that this might be controlled by circadian clocks. It appears that the circadian system aids the effective release and maturation of daily sperm batches, allowing an accumulation of fertile sperm in the male storage organs (Syrova et al. 2003). Overnight retention of sperm in the UVD of the African cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) has been correlated with the nighttime secretion of glycoproteins from the UVD epithelium and concurrent acidification of the UVD lumen by the action of V-ATPase (Bebas et al. 2002a). Similar events related to sperm maturation during retention in UVD might be occurring in *S. litura*.

Sperm was present in the reproductive system of 0–1 d old males, which suggests that sperm release might have occurred in the pupal stage. Similar findings were reported by Polanska et al. (2009) who stated that sperm release started 2 d pre-emergence and coincided with a significant decrease in hemolymph ecdysteroids levels in *S. littoralis*. Quantitative analysis of sperm descent in our study of 0–1 d old males showed 183 and 306 eupyrene sperm bundles to be present in the duplex during the photophase and scotophase, respectively. This number was lower than sperm produced in the testes (3,169) of 0–1 d old males (Table 1a). The number of sperm in the duplex further increased with time at the rate of 140–160 eupyrene sperm bundles with every 24 h rhythmic cycle. However, the number of sperm remained constant in the UVD and SV during every 24 h rhythmic cycle in the present study. There was an alteration in retention of sperm numbers in the UVD and SV during the rhythmic cycle of sperm release in the present investigation on *S. litura*. For instance, during the photophase there were 12 eupyrene sperm bundles in the UVD and 54 in the SV of 0–1 d old males, whereas during the scotophase the number of eupyrene sperm bundles increased to 80 in the UVD and decreased to 14 in the SV of 0–1 d old males.

Our study showed that the pattern of sperm rhythm in P males irradiated with 130 Gy was slightly erratic in comparison with 100 Gy irradiated males. For instance in 1–2 d and 2–3 d old P males irradiated with 130 Gy, sperm number in the UVD decreased by 16.3% during the scotophase in comparison with non-irradiated control males. This could be attributed to a slower release of sperm from the testes caused by irradiation. The numbers of sperm that accumulated in the duplex of P males that were treated with either 100 or 130 Gy were lower in comparison with non-irradiated control males. This led to the conclusion that radiation resulted in an early halt of sperm transfer from the SV to the duplex. The reduction in number of sperm produced in the testes of F_1 adults might be correlated with the reduction in number of sperm that descended from the testes down the reproductive tract during the photophase and the scotophase.

Overall, a comparison of the patterns and amounts of sperm descending during the photophase and the scotophase of P males treated with either 100 or 130 Gy and F_1 males indicated that the negative effect of irradiation on sperm descent was more pronounced in the scotophase than in the photophase.

SPERM ACTIVATION

Physiologically, the highly motile apyrene sperm seems to act as micro stirring bars to facilitate the dissociation of eupyrene sperm bundles in spermatophores after copulation (Happ 1992). Hence, it is important that the apyrene sperm of sub-sterilized males and their F_1 progeny retain their motility in order to ensure that the eupyrene sperm maintain their viability and competence after mating. In our study, sperm became active during the first min of incubation with the natural activator and attained peak activity after 30 min in non-irradiated virgin and mated *S. litura* males. These findings corroborate those of sperm activation in other insects, i.e., sperm became active after 2 min in the bedbug, *Cimex lectularius* Latreille (Hemiptera: Cimicidae) (Davis 1965), 90 s in the Chinese (oak) tussah moth, *Antheraea pernyi* (Guerin-Meneville) (Lepidoptera: Saturniidae) and 5–8 min in the Cecropia moth, *Hyalophora cecropia* L. (Lepidoptera: Saturniidae) (Shepherd 1974b).

Similarly, the time of onset of sperm activation was not negatively affected in P males irradiated with various doses in the range of 100–250 Gy and their F_1 male progeny, whereas with greater sub-lethal doses of radiation, the onset of sperm activation was delayed. Furthermore, the maximum levels of sperm activity were similar in sperm of P males irradiated with sub-sterilizing doses and their F_1 male progeny, while the levels of maximum sperm activity were significantly reduced by radiation treatments in the range of 200–400 Gy.

The velocity of spermatozoa can also be an important factor in sperm competition if more than one male copulates with the same female in quick succession (Birkhead et al. 1999). In an extensive study conducted by Hughes & Davey (1969), the tail beat frequency of the spermatozoa of the American cockroach, *Periplaneta americana* L. (Blattodea: Blattidae) was nearly twice as great in spermatozoa removed from spermathecae (1,050 undulations/min) of females as those removed from the seminal vesicles (600 undulations/min) of males. Similarly, in the present study sperm of non-irradiated virgin males showed a peak intensity of approximately 1,030 undulations/min, whereas the sperm of mated non-irradiated males showed a peak intensity of approximately 900 undulations/min.

Activation of sperm motility involves structural and metabolic changes of the spermatozoa, or it involves chemical stimuli, which lead to the initiation of motility. In the male silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), secretions of the glandula prostatica, which contain an endopeptidase called initiatorin, trigger a cascade of reactions in the apyrene sperm (Osanai et al. 1987a, b). Sperm motility probably results from selective degradation of the glycoprotein by an Arg-C endopeptidase, and then deposition of cAMP on the surface of the cell membrane or in the microslits (Osanai et al. 1991). Similar biochemical cascades in S*. litura* might also have occurred in this study when sperm from the duplex and secretion from the prostatic part of the male reproductive tract were incubated for inducing in vitro sperm motility.

Effect of Gamma Radiation on Sperm Activity

The results of the present study indicate that sperm activity was not inhibited by irradiation, even at the higher doses. The in vitro sperm activation study revealed that the temporal pattern of the proportion of sperm becoming active and the intensity of sperm activity in virgin and mated males were not affected by irradiation doses of either 100 or 130 Gy. Negative effects became apparent when the doses were increased to either 200 or 250 Gy and the effects became drastic with

irradiation doses of either 300 or 400 Gy. It was interesting to note that the proportion of active sperm observed in mated sub-sterilized males was greater than that in unmated sub-sterilized virgin males; whereas the reverse was seen when higher sterilizing doses had been applied. In contrast, the intensity of sperm activation in sub-sterilized and sterilized males was slightly reduced due to mating. Our findings were in close agreement with those of Souka et al. (1975) who reported reduced sperm motility in the tobacco budworm, *Heliothis virescens* F. (Lepidoptera: Noctuidae) irradiated with doses of in the range of 200–400 Gy. The peak intensity (number of undulations/s) of sperm in sub-sterilized male *S. litura* was slightly reduced in comparison with the non-irradiated controls, but peak intensities (undulations/s) of sperm in males that had been irradiated with doses in the range of 200–400 Gy were significantly reduced. The temporal profile of sperm activity in F_1 males was affected by irradiation of their P fathers both in virgin and mated F_1 males in comparison with non-irradiated control males. The present study showed that sperm vigor assessed in terms of levels of sperm motility in virgin and mated males were acceptable for the sub-sterilizing doses of 100 and 130 Gy.

SPERM TRANSFER

Mating success of non-irradiated males with non-irradiated *S. litura* females—as assessed by successful sperm transfer to female spermathecae—was 96.4% and it resulted in 91.4% egg hatch. The numbers of apyrene and eupyrene sperm transferred to the spermathecae were reduced in comparison with the numbers of sperm transferred to the spermatophore at the time of copulation (Seth et al. 2002a). The present study demonstrated that the ratio of apyrene to eupyrene sperm was reduced in the spermathecae, which might indicate that degradation of the apyrene sperm is responsible for inducing the activity of the eupyrene sperm, egg maturation and oviposition (Holt & North 1970; White et al. 1975; Katsuno 1978; Marcotte et al. 2003).

The results presented in this paper demonstrated that *S. litura* males irradiated with either 100 or 130 Gy and their F_1 male progeny were able to transfer adequate complements of eupyrene and apyrene sperm to the female's spermathecae. Successful sperm transfer was correlated with successful mating and the resulting level of induced sterility caused by the amphimixis of oocytes with genetically altered sperm. In the present study, 82–86% of successful matings involving P males and F_1 males resulted in 70–80% sterility in matings of F_1 males in comparison to 50–55% sterility in matings of P males (Table 2). Therefore, the amount of sperm transferred by irradiated males and their F_1 male progeny might be factor in the competitiveness of the sterile males.

Male accessory glands secretory products transferred along with sperm during mating are presumably involved in sperm transfer, maintenance of sperm viability, enhancing oogenesis and triggering of egg laying (Gillott 1996; Gillott 2003; Jin & Gong 2001; Avila et al. 2011). It seems that the radiation-induced changes in the ejaculates of *S. litura* males transferred to females were not enough to disturb the mating competence of the irradiated P males and their F_1 sons.

Sperm behavior in lepidopteran pests, which have sperm dichotomy, is important for the success of the SIT/IS, because this technology can only be effective when the irradiated eupyrene sperm fertilize the normal ova of pest lepidopterans in the field. Moreover, slight changes in quality (viability) of irradiated apyrene and eupyrene sperm may induce the wild females to engage in multiple matings. Sub-sterilizing radiation doses of 100 and 130 Gy did not induce physiological changes in sperm dynamics that affect the reproductive fitness of treated P *S. litura* males or of their F_1 generation male progeny. Understanding the sperm dynamics may help to identify the optimum radiation dose that will not substantially impair the efficacy of the IS technique. The sperm dynamics presented in this paper indicate that the proposed sub-sterilizing doses of 100 and 130 Gy can be considered as suitable for inducing IS for use against *S. litura* pest populations in fields of crops.

Acknowledgments

This work was part of the FAO/IAEA Coordinated Research Project on Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control, and the authors gratefully acknowledge the financial assistance of the IAEA it through research project, RC-15557.

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